SHORT REPORT

Complete germline deletion of the *STK11* gene in a family with Peutz–Jeghers syndrome

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Peutz–Jeghers syndrome (PJS, MIM175200) is an autosomal-dominant inherited disorder characterised by multiple gastrointestinal hamartomatous polyps, melanin spots of the oral mucosa and digits, and an increased risk for various neoplasms. The PJS results from germline alterations of the *STK11/LKB1* tumour suppressor gene, located on 19p13.3, and encoding a serine/threonine kinase. The detection of *STK11* germline mutations, in only 50–70% of PJS families, has suggested a genetic heterogeneity of the disease. We report the case of a family with typical features of PJS, including gastrointestinal hamartomatous, breast cancers and melanin spots of the oral mucosa. Quantitative multiplex PCR of short fluorescent fragments (QMPSF) of the 19p13 region allowed us to identify an approximately 250 kb heterozygous deletion removing entirely the *STK11* locus. This report, which constitutes the first description of a complete germline deletion of *STK11*, shows that the presence of such large genomic deletions should be considered in PJS families without detectable point mutations of *STK11*.

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Introduction

Peutz–Jeghers syndrome (PJS) is a rare autosomal-dominant disease associated with predisposition to benign and malignant tumours. The incidence has been estimated as one in 100 000–200 000 births.^{1–3} Patients present mucocutaneous pigmented macules, especially on the lips, buccal mucosa and digits, diffuse gastrointestinal hamartomatous polyposis and an increased risk of early-onset cancers, such as gastrointestinal carcinoma, breast cancer, genital tract tumours and pancreatic cancer.⁴ The gene

involved in PJS was mapped to chromosome 19p13.3^{5,6} and was shown to be *STK11/LKB1*.^{7,8} The *STK11* gene contains 10 exons spanning 23 kb, and encodes an ubiquitously expressed 433 amino-acid serine threonine-kinase, which might be involved in G1 cell cycle arrest,⁹ p53-dependent apoptosis pathway,¹⁰ vascular endothelial growth factor regulation,¹¹ and in the Wnt – beta catenin signalling pathway.¹² Most of the *STK11* mutations detected in PJS patients result in truncated proteins with incomplete catalytic domains without kinase activity.^{7,8,13,14} PJS represents, therefore, the first cancer susceptibility syndrome resulting from germline inactivation of a kinase activity. The formation of hamartomas and cancers, in *STK11* germline mutation carriers, results from the somatic inactivation of the wild-type allele, according to the Knudson model for tumour suppressor genes inactivation.^{5,15} Germline mutations of the *STK11* gene

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can be detected in only 50–70% of PJS families^{7,16–18} and in 12–50% of individuals with sporadic PJS,^{16,19} which may reflect the involvement of other unidentified genes in PJS. Indeed, PJS families, not linked to chromosome 19p13.3, have already been reported.^{20,21} The alternative hypothesis, which might explain the absence of detectable *STK11* germline mutations in some PJS families, is the existence of alterations, such as genomic rearrangements, which are missed by conventional screening methods.

We report here, for the first time, the detection of a complete genomic deletion of the *STK11* locus in a family with PJS.

Patients and methods

Patients

The proband, born in 1987, was a male from nonconsanguineous parents and the youngest of three siblings. He was initially referred to our department of genetics for learning disabilities and scoliosis. Examination revealed 'Café-au-lait' spots and led to the diagnosis of neurofibromatosis type 1, which was confirmed by the presence of Lisch nodules. At the age of 13 years, he presented an occlusive syndrome with rectal prolapse in relation with a large (5 × 4 cm) sigmoid polyp. The existence of a lentiginosis of the inferior lip led us to suspect a PJS, which was confirmed by pathological examination of the polyp. The father presented 'café au lait' spots and a thoracic neurofibroma, which was consistent with the paternal origin of neurofibromatosis type 1. The mother of the index case had developed, (i) at the age of 43 years, recurrent hamartomatous polyps and a colorectal cancer revealed by bleeding, (ii) at the age of 44 years, a right-breast carcinoma and, (iii) at the age of 45 years, a left-breast carcinoma. The maternal uncle, presented hamartomatous polyps of the colon and colorectal cancer at the age of 43 years. Re-examination of the polyps developed in the mother and the maternal uncle, after the diagnosis of PJS in the index case, confirmed that these polyps were consistent with the diagnosis of PJS. Therefore, the proband had simultaneously an NF1 from paternal origin and a PJS from maternal origin.

Microsatellite analysis

The centromeric and telomeric microsatellite markers *D19S886* and *D19S883*, respectively, located 180 kb upstream and 234 kb downstream of *STK11*, were PCR amplified from genomic DNA using dye-labelled primers and analysed on an automated sequencer (Applied Biosystems) using the Gene scanner Model 672 Fluorescent Fragment Analyzer (Applied Biosystems).

Fluorescence. *in situ* hybridization (FISH) analysis

The RP11-75H6 BAC, obtained from the University of Bari, Italy (<http://www.biologia.uniba.it/rmc>), was labelled

using the Nick translation Reagent Kit (Vysis) and fluorescein-labelled dUTPs (Vysis). Metaphase chromosome spreads and probe were denatured at 72°C for 1 min, then hybridized at 37°C overnight. Chromosome counterstaining was performed with 0.1 µg/ml 4',6-diamidino-2-phenylindole (DAPI) in antifade solution. Slides were visualized on a Nikon-Microphot-FXA microscope and the images were captured using the ISIS digital FISH imaging system (Metasystem).

Quantitative multiplex PCR of short fluorescent fragments (QMPSE)

Short exonic fragments, corresponding to the 10 exons of *STK11* and to the exon 13 of *HMBS/PBGD* located on chromosome 11, used as control, were simultaneously amplified in a first QMPSE. To examine the extent of the deletion, we designed a second QMPSE including exon 1 of *WDR18*, exon 5 of *MIDN*, exon 18 of *DAZAP1* and exon 18 of *MLH1*, used as control. Sequences of primers are indicated in Table 1.

Results

The remarkable association of NF1 and PJS features in the same patient led us initially to test the involvement of the *STK11* gene within this family. Therefore, we analysed first the segregation of the *D19S886* and *D19S883* microsatellite markers, surrounding the *STK11* locus, in the index case, the mother and maternal uncle whose clinical presentation was consistent with PJS. Unexpectedly, this analysis showed an absence of maternal contribution of *D19S886* in the proband, suggesting therefore a 19p13.3 heterozygous deletion inherited from his affected mother (data not shown). Chromosome analysis performed on cultured peripheral blood lymphocytes from the proband, using standard R-banding, revealed no alteration at the 400 band level. FISH analysis, performed with a genomic probe prepared from the RP11-75H6 clone, revealed an asymmetric signal between the two chromosomes 19, which indicated that this BAC overlapped the deleted region (data not shown). In order to confirm the heterozygous deletion of *STK11* and to map precisely the deletion, we used QMPSE, a method based on simultaneous amplification of multiple short sequences under quantitative conditions.²² QMPSE, performed in the proband, his mother and maternal uncle, confirmed the deletion and revealed that this deletion removed all the exons of *STK11* (Figure 1a). In contrast, the telomeric *WDR18*, and centromeric *DAZAP1* and *MIDN* genes were not deleted (Figure 1b).

Discussion

We document here for the first time, in a PJS family, using microsatellite, FISH and QMPSE analysis, a complete heterozygous deletion of the *STK11* locus. Results from

Table 1 Primers used for the QMPSF^a

Gene	Exon	Sense primer ^b	Antisense primer ^c	Size of the amplicon ^d
STK11	1	5' CGAGCTGATGTCGGTGGGT 3'	5' ACAGCGTCTCCGAGTCCAG 3'	193
STK11	2	5' ATCATCCTGACGTTGGGTGCG 3'	5' GGAGACGGGAAGAGGAGCAG 3'	248
STK11	3	5' CCCTCCAGAGCCCCTTTT 3'	5' CACGCTGTCCAGCATTTC 3'	161
STK11	4–5	5' CGGTGGCACCTCAAAT 3'	5' GACCCAGCCGACCAGAT 3'	267
STK11	6	5' TGGGTCCAGAGGACACTCCC 3'	5' CCCTTCCCGATGTTCTCAA 3'	291
STK11	7	5' CCGGCTTCTCCTCAGGGAT 3'	5' TCTAGCGCCCGCTCAACC 3'	174
STK11	8	5' CTGCTTCTGGGCGTTTGC 3'	5' ACCGTGAAGTCCTGAGTGTAGAT 3'	220
STK11	9	5' CTTGCCGCTCCCTCCCA 3'	5' TCCGCCCTGGATTGTTGGT 3'	151
STK11	10	5' GAGTCCGGTAGCCCCATGA 3'	5' TGGTCCGCACAGAAGCATG 3'	237
WDR18	1	5' ATGTGGAGCTGCATCGTGT 3'	5' CCCAGGCGCTGATGTAATT 3'	169
MIDN	5	5' CCGTTCCGCACTGACACT 3'	5' CACGGGGGAGGCTATGCT 3'	181
DAZAP1	11	5' TTACGGGCAGGACTTGAGTGG 3'	5' GTCACGAGTTCACAAGTTTGG 3'	253
MLH1	18	5' GTAGTCTGTGATCTCCGTTT 3'	5' ATGTATGAGTCTCTGCTCTA 3'	266
HMBS	13	5' TAGACGGCTCAGATAGCATACAAG 3'	5' ATGCCTACCAACTGTGGGTCA 3'	208

^aSequences and location of primers were based on the chromosomes 19 and 2 working draft sequences (Build 33).

^bCarry the 5' extension: CGTTAGATAG and are 5' (6-Fam) end labelled.

^cCarry the 5' extension: GATAGGGTTA.

^dIn bp.

Note: PCR were performed in a final volume of 25 μ l containing 100 ng of genomic DNA, 0.15–1 μ M of each primer, 200 μ M dNTPs, 1.5 mM MgCl₂, 10% of DMSO and 1 U of *Taq* DNA polymerase (Abgene). The PCR consisted of 22–25 cycles of 94°C for 15 s, 50°C for 15 s and 72°C for 15 s, preceded by an initial denaturation step of 5 min at 94°C and followed by a final extension of 5 min at 72°C. PCR products (1 μ l) were resuspended in a mix containing 1.25 μ l of deionised formamide, 0.5 μ l of GeneScan-500 Rox (PE Applied Biosystems, Foster City, CA, USA) and 1 μ l of loading buffer. After denaturation for 3 min at 95°C, 2 μ l of each sample was loaded onto a 6% denaturing polyacrylamide gel (Sequagel). Electrophoresis was performed for 3 h on an Applied Biosystems model 377 automated sequencer (PE Applied Biosystems, Foster City, CA, USA). Data were analysed using the Gene Scanner Model 672 Fluorescent Analyser (PE Applied Biosystems, Foster City, CA, USA).

the microsatellite markers and QMPSF analysis allowed us to locate the telomeric breakpoint between *WDR18* exon 1 and *D19S886* and the centromeric breakpoint between *STK11* exon 10 and *MIDN* exon 5 (Figure 2), and to estimate the size of this 19p13.3 deletion between 220 and 250 kb. According to the chromosome 19 draft sequence (<http://www.ncbi.nlm.nih.gov/LocusLink>), this deletion

also removed, in addition to *STK11*, the *GPX4*, *POLR2E*, *HA-1*, *CNN2* and *ABCA7* genes encoding, respectively, the glutathion peroxydase-4, the subunit E of DNA-dependant polymerase II, a minor histocompatibility antigen, the calponin 2 and an ATP-binding cassette protein (Figure 2).

To date, approximately 100 different mutations of *STK11* have been described in PJS patients (Human Gene Mutation Database: <http://archive.uwcm.ac.uk/uwcm/mg/hgmd0.html>). Most mutations are nucleotide substitutions and small insertions or deletions. At the present time, four partial genomic rearrangements of *STK11* have been detected: a deletion of the end of intron 6 and the majority

Figure 1 Detection and characterization of the boundaries of the 19p13 deletion using QMPSF. The Y-axis displays fluorescence in arbitrary units, and the X-axis indicates the size in bp. (a) Deletion of exons 1–10 of *STK11*. The electropherograms of the index case (in blue) and of the affected mother (in green) were superimposed to that of the unaffected father (in red), used as control, by adjusting to the same level the peaks obtained for the control amplicon (*HMBS* exon 13). Heterozygous deletion of the *STK11* exons is visualised by a 50% reduction of the corresponding peaks height. The same profile was obtained for the affected uncle. (b) Absence of deletion of the telomeric *WDR18* and, of the centromeric *MIDN* and *DAZAP1* genes. The electropherogram of the index case (in black) was superimposed to that of the unaffected father (in red) by adjusting to the same level the peaks obtained for the control amplicon (*MLH1* exon 18).

Figure 2 Schematic representation of the 19p13.3 deletion removing the *STK11* locus. CEN and TEL indicate the centromeric and telomeric sides. Genes are indicated above the line and microsatellite markers and the BAC clone used for the FISH analysis below. Grey boxes show the positions of QMPFS amplicons. The deleted regions are designed by white boxes and nondeleted regions by black boxes. Del: deleted. The minimal and maximal estimated sizes of the deletion are indicated by arrows.

of exon 7,¹³ a complex rearrangement including a deletion of exons 4–5 and an inversion of exons 6–7 detected by long-distance PCR,⁸ a deletion of exons 2–7 and a part of exon 8²³ revealed by Protein Truncation Test and a 2 kb deletion detected by Southern blot.¹⁶ Our study shows that complete heterozygous rearrangements of *STK11* also occur in PJS. PJS families linked to 19p13 and without detectable mutation of *STK11* have already been described.^{7,16} The presence of such genomic rearrangements, which are missed by conventional screening methods, based on PCR amplification of each exon, should be therefore considered in PJS families without detectable alteration. The QMPFS method, which can detect heterozygous deletions affecting either a single or several exon(s), should allow to estimate the relative contribution of *STK11* deletions in the aetiology of PJS.

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